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2. Patent application number *(The Patent Office will fill in this part)* — 0317988.4 — 31 JUL 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — Professor Jo Milner, Department of Biology, University of York, YORK Y01 5DD

   Patents ADP number *(if you know it)*

   If the applicant is a corporate body, give the country/state of its incorporation — 8590531001

4. Title of the invention — SPLICING VARIANTS

5. Name of your agent *(if you have one)* — Harrison Goddard Foote

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — Quality House, Quality Court, Chancery Lane, LONDON, WC2A 1HT, GB

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Signature	Date
Harrison Goddard Foote	31 July 2003

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SPLICING VARIANTS

Field of the invention

This invention relates to abnormal spliced variants of genes implicated in the inhibition of apoptosis and to the regulation of apoptosis through the targeting of such variants.

Background to the invention

Bcl-2 is an inhibitor of apoptosis. The functions of the Bcl-2 protein include protection against mitochondrial changes associated with apoptosis. This is achieved by inhibiting pro-apoptotic proteins and by preventing mitochondrial permeability transition. Apoptosis can be triggered by release of cytochrome c and other pro-apoptotic components fom the mitochondria: Bcl-2 is believed to inhibit such events. Consistent with these functions the Bcl-2 protein is predominantly localised to the mitochondria. Bcl-2 may also have additional anti-apoptotic functions yet to be described. It may also block mitochondrial-independent pathways involved in apoptosis.

The human Bcl-2 gene encodes mRNA transcripts of (i) 720 nucleotides in length for Bcl-2α and (ii) of 618 nucleotides in length for Bcl-2β (see Figure 1). Bcl-2α and Bcl-2β represent normal, alternatively spliced variants of the same Bcl-2 gene.

Abnormal and/or constitutive expression of functional Bcl-2 can protect mammalian cells from undergoing apoptosis. Such an effect favours continued cell survival and proliferation, and can initiate and/or maintain abnormal and/or cancerous growth.

In colorectal cancer cells evidence for a novel Bcl-2 – p53 axis has been reported for a number of established human colorectal carcinoma cells lines, including the LoVo and SW48 cell lines. Co-pending patent application GB0306148.8 relates to the silencing of Bcl-2 by RNA interference. p53-dependent apoptosis is induced indicating that Bcl-2 constitutively suppresses a pro-apoptotic function of p53 in colorectal cancer cells. Importantly, this pro-apoptotic function of p53 does not require activation of the p53 protein by genotoxic stress or by other means.

There is a need to identify cell growth control targets for treating malignancies in humans and other mammalian species.

Statements of the invention

According to the present invention there is provided a method of regulating apoptosis in a cell, said method comprising targeting an abnormally spliced mRNA or a product thereof.

Preferably the method involves targeting the junctions of mRNA molecules that are abnormally spliced.

Alternatively the method involves targeting a protein product following translation of an abnormally spliced mRNA.

Preferably the method comprises selective silencing of abnormal splice variants of the Bcl-2 gene.

The term 'selectively silencing' is used to indicate that the silencing is specific for the target gene and that there is no interference with normal, endogenous gene expression which might be detrimental to normal non-cancerous cells.

Preferably the method involves the targeting of abnormal splice variants Bcl-2α-591; Bcl-2β-489; and Bcl-2β-420.

More preferably the method involves targeting the mRNA sequence flanking the splice junction between nucleotides 111 and 241 of Bcl-2α-591.

Preferably the method further comprises introducing into a cell containing said gene, an RNA construct having a nucleotide sequence which is homologous to mRNA within said cell wherein said mRNA includes genetic information of the gene element that is abnormally spliced.

RNA interference (RNAi) induces sequence-specific degradation of homologous mRNA and is initiated by the introduction of dsRNA into cells. In mammalian cells RNAi can be achieved using <u>s</u>mall <u>i</u>nterfering dsRNAs (siRNAs), preferably up to 30 nucleotides long and more preferably 21-22 nucleotides long.

The term 'homologous' is used to indicate at least 50%, preferably 85%, more preferably 90%, and more preferably 95% and most preferably 100% homology to the reference nucleic acid sequence.

The present invention relates to the discovery of abnormal splice variants of Bcl-2 mRNA in human colorectal carcinoma cells. Sequence alignments are given in Figure 1. The novel splice junctions conserve the normal triplet framing of the spliced mRNA products and the functional BH1, BH2, BH3 and BH4 domains of the Bcl-2 protein are also conserved.

Abnormal alternatively spliced variants of Bcl-2 may function constitutively to suppress apoptosis in human and other mammalian cells, enabling abnormal cell survival and abnormal cell proliferation. The expression of abnormally spliced variants of Bcl-2 may thus represent a key oncogenic event in the development of cancer. The abnormal splice junctions of the Bcl-2 mRNA molecules represent selective targets for intervention via RNA interference or other means. The mRNA sequence at these abnormal splice junctions is not present in the normally spliced Bcl-2 mRNAs.

These abnormal Bcl-2 mRNA transcripts are shorter than the full length 'wild type' Bcl-2 mRNA. In contrast analysis of the genomic Bcl-2 by PCR amplification gives the predicted length for wild type Bcl-2 DNA (Figure 2). This indicates that the shorter abnormal Bcl-2 mRNA transcripts are indeed generated by alternative splicing of RNA, rather than genomic events with loss of DNA coding sequence from the human Bcl-2 gene.

The abnormal alternative spliced variants of Bcl-2 expressed in human colorectal cancer cells retain all known functional domains of the protein (see Figure 1) and are functional in the suppression of apoptosis. Functionality is also evident in colorectal carcinoma cell lines in which Bcl-2 expression appears to comprise solely of the abnormal alternative spliced form(s). In such cells the selective silencing of Bcl-2 expression by RNA interference induces apoptosis (e.g. LoVo cells; Jiang and Milner, 2003; note that normal full length mRNA for Bcl-2α nor for normal full length Bcl-2β mRNA cannot be detected in LoVo, SW48 or in HCT116 cell lines).

Selective silencing of alternatively spliced Bcl-2 expression may be achieved by RNA interference, or by any other 'silencing means' such as small molecules, peptides and/or related molecules which inhibit Bcl-2, either directly or indirectly, and also Bcl-2 derived products including abnormal Bcl-2 splice variants. Anti-sense RNA, shRNA, miRNA and any other RNA and/or DNA based strategies may also be used. Tumour cells other than colorectal cancer cells may similarly be treated.

In one embodiment the present invention provides a nucleotide construct with a nucleotide sequence which is homologous to mRNA transcribed from an abnormally spliced gene.

Preferably the nucleotide construct comprises dsRNA. Preferably the construct is 30 or less nucleotides long. More preferably the RNA construct is 20 to 30 nucleotides long. Most preferably the RNA construct is 21 to 22 nucleotides long.

In one embodiment the invention provides a nucleotide construct such as anti-sense RNA, shRNA or miRNA as means for silencing the expression of an abnormally spliced gene for use as a medicament.

In an alternate embodiment the invention provides a small molecule or protein which interacts with or binds with a protein expressed by an abnormally spliced mRNA for use as a medicament.

In an alternative embodiment the invention provides a nucleotide construct such as anti-sense RNA, shRNA or miRNA for the manufacture of a medicament for the treatment of cancerous cell growth.

In an alternate embodiment the invention provides a small molecule or protein which interacts with or binds with a protein expressed by an abnormally spliced mRNA for the manufacture of a medicament for the treatment of cancerous cell growth.

The invention also provides a pharmaceutical composition comprising a nucleotide construct such as anti-sense RNA, shRNA or miRNA and a pharmaceutically acceptable diluent or carrier.

In an alternate embodiment the invention provides a small molecule or protein which interacts with or binds with a protein expressed by an abnormally spliced mRNA and a pharmaceutically acceptable diluent or carrier.

Detailed Description of the Invention

The present invention will now be described by way of example only and with reference to the following diagrams;

Figure 1

Sequence alignments of human Bcl-2 splice variants in colorectal cell lines (including LoVo; SW48 and HCT116). Boxed areas indicate functional domains of Bcl-2. Note that Bcl-2α-591 and Bcl-2β-489 retain all functional domain sequences. Dashes indicate missing sequences from abnormally spliced Bcl-2 variants.

Figure 2

Sizing of Bcl-2 genomic DNA following PCR amplification from individual human colorectal cell lines as indicated, using primers designed to span all abnormal splice sites identified to date. The predicted size for the intact genomic Bcl-2 DNA PCR-generated sequence, using the chosen primers, is 570 base pairs. This is the size observed in all colorectal cell lines tested to date, as indicated on the gels. [Note that genomic Bcl-2 is normally only spliced to generate the Bcl-2α and Bcl-2β variants].

Figure 3

Expression of abnormal alternatively spliced variants of human Bcl-2 in vitro and immunoprecipitation with anti-Bcl-2 antibodies. Bcl-2 mRNA from human colorectal cancer cells was reverse transcribed to produce a cDNA template from which cRNA was transcribed and translated. Translation was performed in the presence of 35S-methionine and radiolabelled protein was visualised by autoradiography following

immunoprecipitation and resolution by SDS-PAGE. Three abnormal splice variants are shown (Bcl-2α-591; Bcl-2β-489; and Bcl-2β-420 as indicated).

Cloning and expression of abnormal alternative splice variants of Bcl-2 in vitro.

Abnormal alternative splice variants of Bcl-2 mRNAs have been cloned from colorectal cancer cells and expressed in vitro. The results demonstrate that the abnormal alternative spliced variants of Bcl-2 are expressed as protein (Figure 3).

Lack of specific Bcl-2 epitopes was observed for the protein products encoded by the abnormal alternatively spliced Bcl-2 variants. Abnormal splicing in some way interferes with epitope availability for antibody recognition. It is proposed that epitope loss may prove to be a useful indicator of alternatively spliced Bcl-2 expression. For example, the variant Bcl-2α-591 contains a novel splice junction between nucleotides 111 and 241 (Figure 1): the protein expressed endogenously from this splice variant in human cells reacts poorly with the N19 anti-Bcl-2 antibody in immunoblots (Jiang and Milner, 2003), and in immunoprecipitation reactions following its expression in vitro (Figure 3). Loss of antibody reactivity may also be evident in tissue sections stained by immunocytochemistry. Epitope loss or modification may prove to be of clinical and diagnostic importance for identifying the expression of abnormal alternative spliced variants of Bcl-2 in human tissues. The same prinicples apply to tissues of other mammalian species.

Alternative abnormal spliced variants of Bcl-2 may represent a tumour-related abnormality. This abnormality may not be restricted to cancers arising from

colorectal epithelial cells. Other tumour types may also be affected, including other epithelial tumours and/or tumours/malignancies arising from other cell types. Any tumour-related abnormality represents a promising target for selective therapy designed to selectively target malignancies in humans and in other mammalian species. Such therapies may, in principle, be designed to suppress gene expression using, for example, RNA interference. An alternative approach would be to target functional protein-protein interactions by, for example, small molecules designed to disrupt essential molecular interfaces between the Bcl-2 protein and its functional protein partners. Any differences in protein structure created as a result of abnormal alternative splicing of Bcl-2 mRNA represent potential tumour-specific targets for novel anti-cancer molecules and/or other reagents.

References:

1. **Jiang M & Milner J.** Bcl-2 constitutively suppresses p53-dependent apoptosis in colorectal cancer cells. **Genes & Development,** 17; 832-837 (2003).

Figure 1

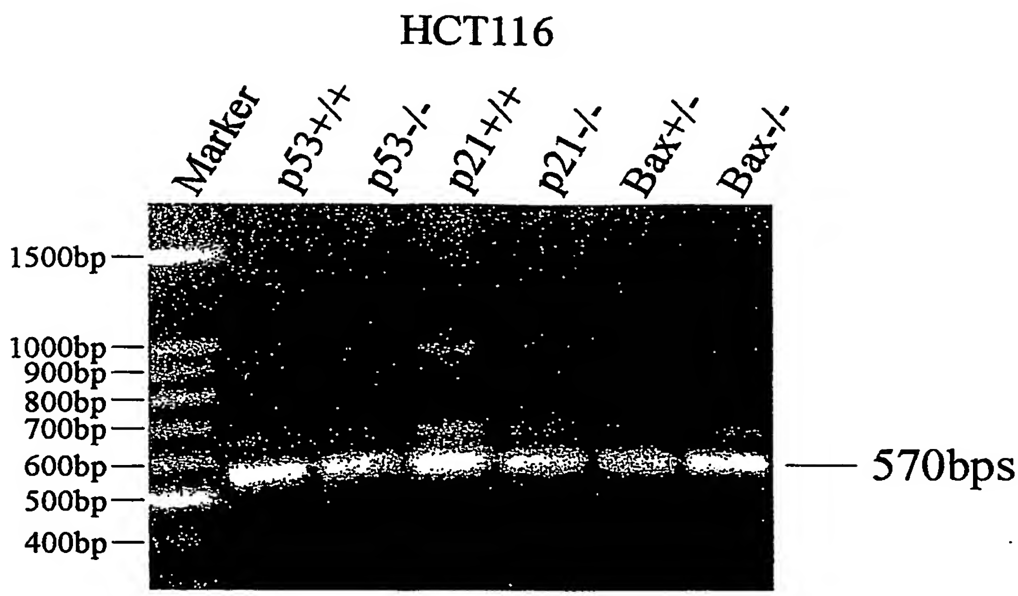
```
Bcl-2α       atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2α-591   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2α-588   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2α-480   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2α-633   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2β       atg gcg cac gct ggg aga acg ggg tac gac aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl 2β 489   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2β-474   atg gcg cac gct ggg aga acg ggg tac gac aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75
Bcl-2β-420   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg --- ---  69
Bcl-2β-315   atg gcg cac gct ggg aga aca ggg tac gat aac cgg gag ata gtg atg aag tac atc cat tat aag ctg tcg cag  75

Bcl-2α       agg ggc tac gag tgg gat gcg gga gat gtg ggc gcc gcg ccc ccg ggg gcc gcc ccc gcg ccg ggc atc ttc tcc 150
Bcl-2α-591   agg ggc tac gag tgg gat gcg gga gat gtg ggc gcc --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl-2α-588   agg ggc tac gag tgg gat gcg gga gat gtg ggc gcc --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl-2α-480   agg ggc tac --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  84
Bcl-2α-633   agg ggc gcg gcg gtg gtc gag acc aga acg gcc ttt cca agg gcg gcg gcg gcg gtt aca aca gct acg gtg gtt 150
Bcl-3β       agg ggc tac gag tgg gat gcg gga gat gtg ggc gcc gcg ccc ccg ggg gcc gcc ccc gca ccg ggc atc ttc tcc 150
Bcl-2β-489   agg ggc tac gag tgg gat gcg gga gat gtg ggc gcc --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl-2β-474   agg ggc cac gag tgg gat gcg gga gat gtg ggc gcc gcg ccc ccg --- --- --- --- --- --- --- --- --- --- 120
Bcl-2β-420   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  69
Bcl·2β-315   agg --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  78

Bcl-2α       tcg cag ccc ggg cac acg ccc cat aca gcc gca tcc cgg gac ccg gtc gcc agg acc tcg ccg ctg cag acc ccg 225
Bcl-2α-591   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl-2α-588   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl-2α-480   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  84
Bcl-2α-633   acg gcg --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- 156
Bcl-2β       tcc cag ccc ggg cac acg ccc cat cca gcc gca tcc cgc gac ccg gtc gcc agg acc tcg ccg ctg cag acc ccg 180
Bcl-2β-489   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- 111
Bcl·2β-474   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- 120
Bcl-2β-420   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  69
Bcl-2β-315   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  78

Bcl-2α       gct gcc ccc ggc gcc gcc gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 300
Bcl-2α-591   --- --- --- --- --- gcc gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 171
Bcl-2α-588   --- --- --- --- --- --- gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 168
Bcl-2α-480   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  84
Bcl-2α-633   --- --- --- --- --- --- gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 213
Bcl-2β       gct gcc ccc ggc gcc gcc gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg gcc ctc cgc caa gcc 300
Bcl 2β 489                           gcc gcg ggg cct gcg ctc agc ccg gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 171
Bcl-2β-474   --- --- --- --- --- --- --- --- --- --- --- --- --- gtg cca cct gtg gtc cac ctg acc ctc cgc cag gcc 156
Bcl-2β-420   --- --- --- --- --- --- --- --- --- --- --- --- --- --- cca cct gtg gtc cac ctg acc ctc cgc cag gcc 102
Bcl-2β-315   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  78

Bcl-2α       ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agg cag ctg cac ctg acg ccc ttc acc 375
Bcl-2α-591   ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 246
Bcl-2α-588   ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 243
Bcl-2α-480   --- --- --- --- --- --- --- --- cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 135
Bcl-2α-633   ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 288
Bcl-3β       ggc gac gac ttc tcc cgc cgc tac cgc ggc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 375
Bcl-2β-489   ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 246
Bcl-2β-474   ggc gac gac ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ctc acc 231
Bcl-2β-420   ggc gac ggc ttc tcc cgc cgc tac cgc cgc gac ttc gcc gag atg tcc agc cag ctg cac ctg acg ccc ttc acc 177
Bcl-2β-315   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  78
```

continued

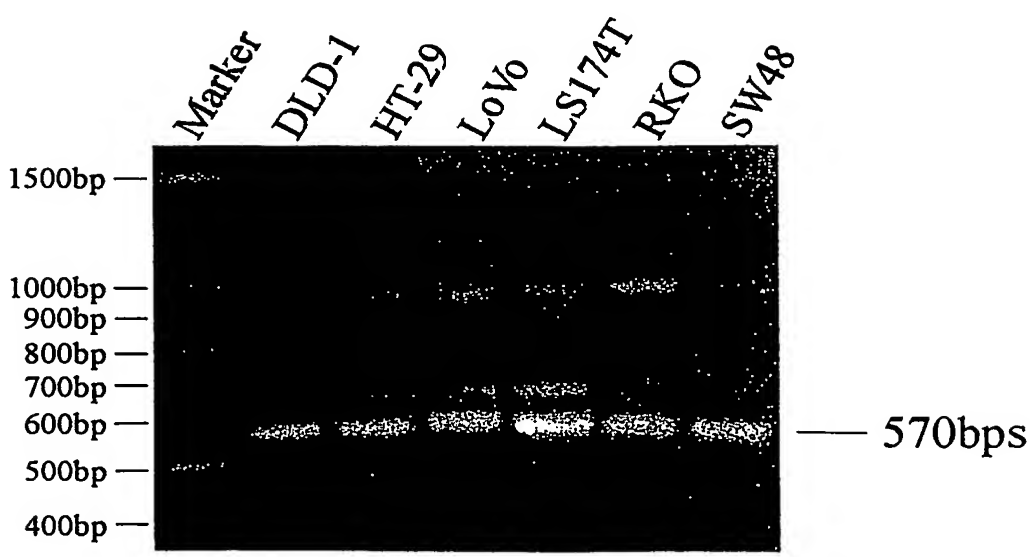
Figure 1

```
Bcl-2α      gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 450
Bcl-2α-591  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 321
Bcl-2α-588  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 318
Bcl-2α-480  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 210
Bcl-2α-633  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 363
Bcl-2β      gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 450
Bcl 2β 489  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 321
Bcl-2β-474  gcg cgg gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 306
Bcl-2β-420  gcg cgg gga cgc ttt gcc tcg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 252
Bcl-2β-315  --- --- gga cgc ttt gcc acg gtg gtg gag gag ctc ttc agg gac ggg gtg aac tgg ggg agg att gtg gcc ttc 147

Bcl-2α      ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 525
Bcl-2α-591  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 396
Bcl-2α-588  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tca ccc ctg gtg gac aac atc gcc ctg 393
Bcl-2α-480  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 285
Bcl-2α-633  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 438
Bcl-2β      ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 525
Bcl-2β-489  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 396
Bcl-2β-474  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gcc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 381
Bcl-2β-420  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 327
Bcl-2β-315  ttt gag ttc ggt ggg gtc atg tgt gtg gag agc gtc aac cgg gag atg tcg ccc ctg gtg gac aac atc gcc ctg 222

Bcl-2α      tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg --- --- --- --- --- 585
Bcl-2α-591  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg --- --- --- --- --- 456
Bcl-2α-588  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg --- --- --- --- --- 453
Bcl-2α-480  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg --- --- --- --- --- 345
Bcl-2α-633  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg --- --- --- --- --- 498
Bcl-2β      tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg gta ggt gca tct ggt 600
Bcl-2β-489  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg gta ggt gca tct ggt 471
Bcl-2β-474  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg gta ggt gca tct ggt 456
Bcl-2β-420  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg gta ggt gca tct ggt 402
Bcl-2β-315  tgg atg act gag tac ctg aac cgg cac ctg cac acc tgg atc cag gat aac gga ggc tgg gta ggt gca tct ggt 297

Bcl-2α      --- --- --- --- --- --- gat gcc ttt gtg gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg 642
Bcl-2α-591  --- --- --- --- --- --- gat gcc ttt gtg gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg 513
Bcl-2α-588  --- --- --- --- --- --- gat gcc ttt gtg gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg 510
Bcl-2α-480  --- --- --- --- --- --- gat gcc ttt gtg gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg 402
Bcl-2α-633  --- --- --- --- --- --- gat gcc ttt gtg gaa ctg tac ggc ccc agc atg cgg cct ctg ttt gat ttc tcc tgg 555
Bcl-2β      gat gtg agt ctg ggc tga 618
Bcl 2β 489  gat gtg agt ctg ggc tga 489
Bcl-2β-474  gat gtg agt ctg ggc tga 474
Bcl-2β-420  gat gtg agt ctg ggc tga 420
Bcl-2β-315  gat gtg agt ctg ggc tga 315

Bcl-2α      ctg tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc ctg ggt gcc tat ctg ggc cac aag 717
Bcl-2α-591  ctg tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc ctg ggt gcc tat ctg ggc cac aag 588
Bcl-2α-588  ctg tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc ctg ggt gcc tat ctg ggc cac aag 585
Bcl-2α-480  ctg tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc ctg ggt gcc tat ctg ggc cac aag 477
Bcl-2α-633  ctg tct ctg aag act ctg ctc agt ttg gcc ctg gtg gga gct tgc atc acc ctg ggt gcc tat ctg ggc cac aag 630

Bcl-2α      tga 720
Bcl-2α-591  tga 591
Bcl-2α-588  tga 588
Bcl-2α-480  tga 480
Bcl-2α-633  tga 633
```

Figure 2



HCT116
Marker
p53+/+
p53-/-
p21+/+
p21-/-
Bax+/-
Bax-/-
1500bp
1000bp
900bp
800bp
700bp
600bp
500bp
400bp
570bps



Marker
DLD-1
HT-29
LoVo
LS174T
RKO
SW48
1500bp
1000bp
900bp
800bp
700bp
600bp
500bp
400bp
570bps

Figure 3


Total
BD
C-2
N19
Ab-1
Ab-2
Ab-4
Do-1
Bcl-2α-591
—16.5kDa
Bcl-2β- 489
—16.5kDa
Bcl-2β-420
—16.5kDa